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REARING OF TEXAS TABANIDAE (DIPTERA). I. COLLECTION, FEEDING, AND MAINTENANCE OF COASTAL MARSH SPECIES

Patrick H. Thompson and Peter C. Krauter

Abstract.—In order to study methods of rearing, 1639 females of several species of coastal marsh Tabanidae were collected in modified Manitoba Traps and in modified Animal Traps, augmented with CO_2 from dry ice, at West Galveston Bay, Texas; five weekly collections from 18 July–17 August, inclusive, produced the following totals of dominant species: *Tabanus nigrovittatus* Macquart, 1228; *T. lineola* var. *hinellus* Philip, 349; and *T. acutus* (Bigot), 62. Immediately after their collection, many females fed on warmed, citrated beef blood which was held in prophylactics derived from animal membranes. From 26.7–39.6% of 353 females of the three species engorged to repletion on prophylactics placed on top of trap collection containers. Survivorship curves for one sample population showed that mortality rates were fairly constant at all age levels for these three species; 50% and 0% survivorships, respectively, occurred at 5 days and 50 days for *T. nigrovittatus*; 4 days and 36 days, *T. lineola* var. *hinellus*; and 9 days and 49 days, *T. acutus*. Comparison of collection dates of minor species, with that from a study made in 1971, indicated that several inland species had greatly declined or disappeared in the 6-year interim between 1971 and 1977.

Study of Tabanidae within the laboratory has been impeded by negative results in rearing these insects. To the present day, scientists throughout the world have attempted to maintain and to perpetuate many species, mostly with refractory results. According to these researchers, major hurdles to such success with tabanids have been caused by the reluctance of these insects to accept blood; by their habit of indiscriminate oviposition in cages, often under conditions not conducive to maturation of the eggs; by the refusal of the larvae to feed or by their cannibalistic voracity, which also hinders rearing en masse; by mortality resulting from physiological disorders during ecdysis and pupation and by disease organisms throughout development; and by the inhibitory effect of cage retention upon reproductive behavior. To complicate this research dilemma, the study of many species has tended to divide the disciplinary research effort, and as a result, confused the interpretation of efforts with individual species.

In conjunction with our studies of feeding behavior and reproductive

physiology, rearing attempts of several species of Coastal Plain Tabanidae were begun in 1977. Initial work with summer tabanids at inland locations was greatly restricted by very small populations of nearly all species, even the most abundant and successful dominants. On the other hand, populations of several species inhabiting marshes along the Gulf of Mexico were apparently unaffected by the factors reducing populations of the inland forms.

The Coastal Marsh Fauna

Inland from the barrier beaches and estuaries of the Texas coast line, are intermittently flooded tidelands inhabited by a specialized fauna of Tabanidae distinct from other faunas of the Coastal Plain found inland, and of other groups of tideland faunas found eastward. Specifically, this fauna is characterized by five species which are intimately associated with coastal marshes. *Tabanus eadsi* Philip and *T. texanus* Hine share the same range along the south Texas coast, a range extending into western Louisiana (Thompson and Pechuman, 1970). A larger, and sometimes more prominent species, numerically, is *T. acutus* (Bigot), a form occurring from West Galveston Bay, and possibly further south, eastward along the Gulf to coastal counties of the Florida Panhandle (Fairchild, 1937; Stone, 1938). The distribution of *T. lineola* var. *hinellus* Philip, of *T. nigrovittatus* Macquart and of their related forms extends eastward along the Gulf Coast, and then northward along the Atlantic Coast (Philip, 1965).

A survey of marshes on the south Texas coast line in 1971 by Thompson (1973) characterized this ecosystem and the tabanid populations then inhabiting it, by presenting the relative abundance and seasonal ranges of the species comprising the fauna; by providing population indices for dominant and subdominant species throughout the season; and by describing the traps used in collecting the insects and in accumulating the data. A concomitant study published soon thereafter (Thompson and Gregg, 1974) described the trap modifications necessary under the rigorous coastal weather conditions; and discussed the relative efficiency of the 2 trap types used. More significantly, this work greatly simplified the objectives of the present study.

Methods and Results

Changes in the fauna.—Initial trap collections on 18 July showed that the population structure of the West Bay study area had changed in the 6-year interim since the previous (1971) study (Thompson, 1973) (Table 1). Although consecutive weekly population indices from 18 July through 17 August showed that *T. nigrovittatus* still dominated the West Bay

Table 1. Relative abundance of *Tabanus* spp. taken in five consecutive weeks during late July and early August, 1971 and 1977, West Bay.

Species	Percent of Population 1971	1977
T. nigrovittatus Macquart[a]	88.5	74.0
T. lineola var. *hinellus* Philip	1.5	21.1
T. sulcifrons Macquart	5.6	0
T. acutus (Bigot)	0.3	3.7
T. subsimilis subsimilis Bellardi	1.7	0.2
T. texanus Hine	1.0	0.1
Remaining species[b]	1.4 (7 spp.)	0.9 (2 spp.)
Total percent	100.0	100.0
Total flies	4,724	1,660

[a] Sens. lat., i.e., nominal *T. nigrovittatus* and *T. nigrovittatus* var. *fulvilineis* Philip were not differentiated for the purposes of this study because of the large number of intermediate specimens observed here.

[b] Species found only in 1971: *Tabanus atratus* F., *T. cymatophorus* Osten Sacken, *T. eadsi* Philip, *T. mularis* Stone, and *T. stygius* Say; in both years, *Tabanus lincola lineola* F. and *Chrysops flavidus* Wiedemann.

marshes during the summer months, 2 coastal marsh species, *T. acutus* and *T. lineola* var. *hinellus*, markedly increased. In addition, inland forms drastically declined or disappeared. No specimens of *T. sulcifrons* were taken although 265 females had been trapped in 1971. Five other inland species represented by 1–10 specimens during the same period in 1971 were not taken in 1977 (q.v., footnote "b", Table 1). These differences become more meaningful considering that the 1977 catches were augmented with CO_2. (The harsh winter temperatures which probably decimated inland populations of these same species, could also have affected those at West Bay.)

Trap characteristics.—Five weekly collections, usually on several consecutive days in each week, were made from 18 July through 17 August. In order to collect large numbers of *Tabanus nigrovittatus* et al., several traps of 2 types were operated in areas previously shown to be productive (Thompson, 1973). The modified Manitoba Trap (MT) and modified Animal Trap (AT) were improved for use under the windy, open and sunlighted conditions of the marsh. In order to reduce heat within the collection containers of the traps and thereby to reduce fly mortality and to enable feeding by the flies held within them, the top walls or ceilings of the containers were replaced by ¼ in.-mesh hardware cloth.

Previous trapping experience with the coastal marsh species at West Bay in 1971 indicated that the AT was more collective than the MT (i.e., it collected more species in larger numbers). Also to advantage, the larger

size of the collection cage easily accommodated the many flies required by the work. Finally, the AT collection container made a more suitable cage for permanent retention of the flies in the laboratory, both because of the dimensions and because of the nontoxic nature of the construction materials (the styrene of MT collection containers is known to show insect toxicity).

Artificial feeding in the field.—Flies were fed in the field immediately after they entered the traps, or soon thereafter, with whole beef blood treated with sodium citrate (10 g/gal). This blood was offered to the insects using animal membranes manufactured from lamb intestine; although this material is commercially available in sheets, using the material in the form of prophylactics allowed the membranes to be used as containers for the blood. Fourex Natural Skins[1] (Schmid Laboratories, Inc., Little Falls, New York 07424) were each filled with 30 ml of citrated beef blood and then closed with an overhand knot near the open end. This quantity of blood was more than ample to feed many flies and also expose a large surface of the prophylactic across the screened ceiling of the collection container. In the field, the blood was warmed on the hot radiator of the vehicle which transported the collector between trap sites.

Before the blood-filled prophylactics were placed on the top of the AT collection cage, the cage was enveloped by a corrugated cardboard box which contained a rectangular opening, 3 in. × 8 in., centered in the ceiling of the box. This opening was intended to serve the same function as the aperture beneath the collection cage in the top of the trap frame—to concentrate the insects by using a positive response to light. Then, when trapped female flies moved to the screened cage surface beneath the opening in the cardboard box, the prophylactics were placed above them on the top of the cage.

Feeding periodicity and optimal time of collection.—Mariculturists working shrimp ponds in the study area offered valuable suggestions on the feeding periodicity of tabanids in the marshes. Considering the primary objective of the work at the time—the collection of avid flies in large numbers, rather than the collection of data on diurnal rhythms—their suggestions were most helpful. These men described predawn attacks by annoying numbers of flies; the men had attempted, then, to avoid the summer sun through an early work day. As we soon discovered, the magnitude of feeding attacks and trap catches from before dawn to midmorning, substantiated their reports and greatly enhanced the efficiency of the work.

The short periods of intense biting activity were most efficiently utilized by feeding each cage of flies after the cage was taken off the trap frame. In this manner, flies were being trapped continuously without interruption.

(Activity at dusk increased over that at mid-day but did not become as intense as that at sunrise.) After removal of each AT cage, the opening in the bottom of the cage—a funnel-shaped baffle made of cellulose acetate—was sealed with wadded tissue paper, and the cage was transported to the lab within an insulated picnic chest having convenient dimensions for this purpose (Thermos Cooler No. 7719[1]; King-Seeley Thermos Co., Norwich, Connecticut 06360). The insulating capacity of these containers was adequate for safe retention of the flies below critically high temperatures, and the insects could be returned to the laboratory in darkness, thereby preventing stress during transport.

Initial handling of the catch.—After collection and return of the catch, flies were knocked down by chilling them in a refrigerator freezer compartment for 10 min. or by anesthesia with N_2. The former method proved more effective for the large cages being used. Knock-down enabled identification, and subsequent separation by species, in order to collect data useful in subsequent trapping and to determine which species were accepting blood and which species laid what eggs. After definitive determination of egg masses later became possible (Thompson and Holmes, 1979), trapped insects were maintained in their respective AT cages until they died.

Feeding success.—During early collections, flies were examined for the presence of blood after anesthesia and identification. At this time, the abdominal segments of heavily engorged specimens were distended and rounded and the brownish integument became blackish. In some specimens not feeding to repletion, the reddish coloration of fresh blood could be recognized in the middle of the abdomen by shining the light from a microscope lamp through it. Additional study of females which were apparently unengorged, showed the presence of blood that was not detected by gross examination or by light transmission; this blood was observed on the tip of a straight pin which was used to puncture the hindgut through the posterior abdominal segments of dead females. Table 2 shows the results of initial (field) feeding by three coastal marsh species taken in collections of three dates.

Engorgement and mortality.—Feeding ratios were observed in three catches but the mortality of engorged vs. unengorged insects was not recorded because of more pressing priorities at the time. Nevertheless, the large percentages of engorged females which failed to survive feeding, transportation, or anesthesia seemed unusual.

Laboratory maintenance of adults.—Flies were initially retained in half-pint ice cream cartons in order to encourage feeding on blood-filled prophylactics and to confine them in a chamber where light intensity and temperature could be controlled. Later, flies were held in cages in the

Table 2. Percentages of *Tabanus* females feeding after collection, three samples, West Bay, 1977.[a]

Species	19 July		26 July		18 Aug.		Totals Fed (+)
	+	−	+	−	+	−	
T. nigrovittatus Macquart[b]	4	2	4	3	1	19	9 (27.3%)
T. lineola var. *hinellus* Philip	18	22	38	72	47	63	103 (39.6%)

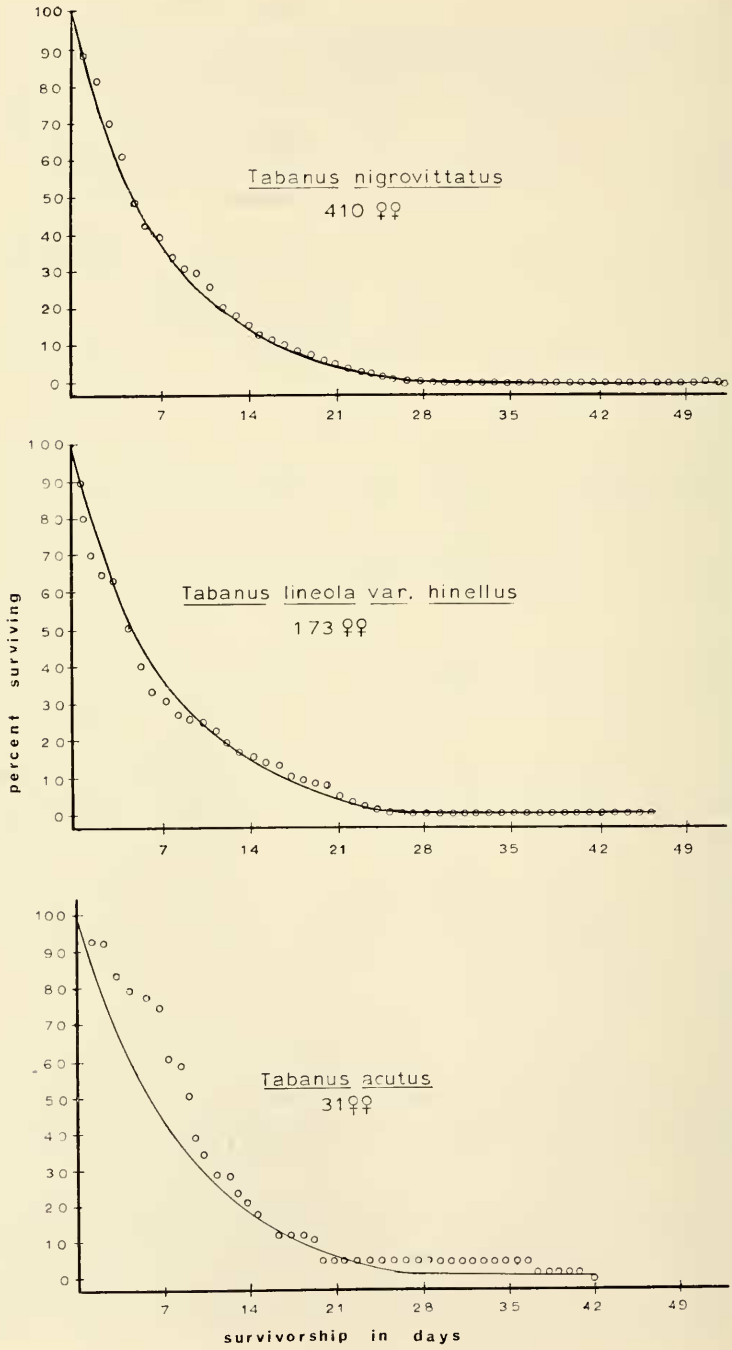
[a] Sixteen of 60 *T. acutus* females (26.7%) taken 9 August fed.

[b] Punch test with a pin showed that an additional 12 females taken on 18 August had fed.

lab when females failed to oviposit in cartons and when the control of light and temperature no longer seemed necessary to feeding or to oviposition. In addition, catches were no longer being anesthetized for identification so that transfer to smaller cages was unnecessary. At this time, then, specimens were retained in AT cages on benches in the lab. Ambient light from two large windows in one wall determined the natural photoperiod of the season. Thermometers and hygrothermographs operated in the lab throughout this period recorded temperatures of 75°F ± 5 and RH of 65% ± 5, respectively. To provide any additional moisture required, 4 in. × 4 in. cotton gauze pads placed atop the cages were continuously saturated with distilled water from an inverted pint Mason Jar above them. Only on rare occasions was a fly observed on or near this surface; therefore, ambient humidity in the room, humidity which was determined in great part by the season, was adequate under the conditions observed here.

A 10% sucrose (cane sugar) solution was provided continuously and blood was offered daily, using the technique previously described, except that prophylactics of blood were heated by immersing them in containers of warm water.

Longevity.—Under the conditions described above, survivorship of specimens taken in one sample is shown in Fig. 1. This figure shows that the rate of mortality was fairly constant at all age levels for the three species considered. This curve corresponds to Slobodkin's Type III curve (Slobodkin, 1962), a plot line becoming straight when the ordinate scale is logarithmic. The relative regularities of the graph lines for these three species reflect, in great part, the corresponding sizes of the sample populations; with *Tabanus nigrovittatus*, a species represented by 410 specimens in this sample, the mortality rate was more constant than with the less numerous species.


Tabanus nigrovittatus
410 ♀♀
Tabanus lineola var. hinellus
173 ♀♀
Tabanus acutus
31♀♀
percent surviving
survivorship in days

Conclusions

The techniques and materials used here for collecting, feeding and maintaining *Tabanus nigrovittatus*, *T. lineola* var. *hinellus* and *T. acutus* will enable further research in rearing these species. Many references considering collection devices for these and other coastal marsh species of eastern North America have been cited by Axtell (1976). Bearing collecting methodology in mind, the present work and that conducted 6 years before (Thompson, 1973), show that the AT and the MT are highly effective for attracting and trapping *T. nigrovittatus* in large numbers. The efficiency of collecting methods for the less numerous species considered here, such as the potential of the MT for taking *T. lineola* var. *hinellus* (Thompson and Gregg, 1974), needs further research.

Feeding of many species of Tabanidae on a great variety of vertebrate hosts has been attempted primarily in conjunction with animal disease transmission experiments. These studies, exhaustively reviewed by Krinsky (1976), have usually dealt with small numbers of insects—numbers necessary only to effect the transfer of pathogens from carriers to a small number of uninfected susceptibles. Because of this priority, rather than the engorgement of large numbers of flies intended for egg production, these studies offer little quantitative information on feeding methods and host acceptance. Moreover, fundamental studies of the basic biology of some species, studies dealing with engorgement and oviposition per se, such as those of Hafez et al. (1970), Jones and Anthony (1964), Roberts (1966), Schwardt (1936), Singh (1967), Webb and Wells (1924) and Wilson (1967), have not offered much insight into the relevant factors which stimulate feeding under conditions of retention and confinement. Also, these studies reported poor feeding results or did not present quantitative information on the numbers of flies exposed to the hosts or the percentages of those numbers feeding. In addition, papers reporting prominent success with artificial methods are very rare. Hafez et al. (1970) noted that *Tabanus taeniola* Palisot de Beauvois females, in contrast to other Egyptian species, fed to repletion on citrated calf blood. During studies of blood meal volume and digestive enzymes, Thomas and Gooding (1976) noted that several species of *Chrysops* and *Hybomitra* accepted warmed defibrinated beef blood through Silverlight membranes.

Results with beef blood-filled prophylactics used in this study were markedly negative or positive. Approximately 25–50 adult females of

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Fig. 1. Survivorship of three coastal marsh tabanids, taken at West Galveston Bay, July and August, 1977.

Tabanus fuscicostatus Hine, *T. lineola lineola*, *T. melanocerus* Wiedemann, *T. proximus* Walker, *T. subsimilis subsimilis* and *T. sulcifrons* each rejected this combination while the same numbers, or more, of *T. mularis*, *T. abactor* Philip and the three coastal marsh species considered here, readily accepted it. In most instances, the insects had come to traps baited with dry ice; and at least some individuals of each of these species were offered blood-filled prophylactics on the tops of trap collection containers very soon after being taken. These data, as well as those of Thomas and Gooding (1976), suggest membranes hold potential success in feeding this group of Diptera, as they have for tsetse species and mosquitoes. On the other hand, results with free blood could be much less favorable; none of the species that we exposed to citrated beef blood on cotton, including *Hybomitra lasiophthalma*, fed.

In studies of the longevity of several hundred caged *Tabanus nigrovittatus* females in the laboratory in Delaware, Olkowski (1966) expressed survivorship in "mean fly days" (12.3 days for *T. nigrovittatus*). Comparing his data with ours is not very meaningful because he excluded 43% of his population sample because these females survived less than 6 days. Secondly, he presents no expression of variation in this population.

In any event, the survivorship that we observed in this the present work, as the collecting and feeding results that we also experienced, will enable study of further consecutive events in the life histories of these species.

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Veterinary Toxicology and Entomology Research Laboratory, Fed. Res., Sci. and Educ. Admin., USDA, College Station, Texas 77840.

Footnote

[1] This paper reports the results of research only. Mention of a commercial or proprietary product does not constitute a recommendation for use by the U.S. Department of Agriculture.